codon83 (Serine83Arginine) was noted in 1 strain having a CIP MIC of 0.012 and a NA MIC of 32 μg/ml. All 28 strains that had a CIP MIC of 0.016—1.5 μg/ml and frank resistance to NA (MIC ≥16 μg/ml; DD zone of inhibition ≤13 mm) carried a transversion mutation in codon 83 of *gyr*A (Serine83Isoleucine). We noted another transversion mutation in codon 85 of *par*C (Serine85Leucine) in 14 of these 28 strains that had a CIP MIC of ≥0.125—1.5 and a NA MIC ≥256 μg/ml). We could not detect any mutations in *gyr*B ($n = 24$) and *par*E ($n = 28$) genes.

Conclusion: Reduced CIP-susceptibility and NA-resistance of *V. cholerae* is associated with a *gyr*A mutation (Serine83Isoleucine). A further decrease in CIP-susceptibility is associated with *par*C mutation (Serine85Leucine).

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17.019

Prevalance of Extended-Spectrum β-Lactamases produced by *Klebsiella spp*. from various clinical samples in an urban Hospital in South India

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Background:Organisms producing extended-spectrum β-lactamases(ESBL) are emerging around the world as a source of resistance to oxyiminocephalosporins such as cefotaxime, ceftazidime and ceftrioxone.This study was undertaken to study the prevalence of ESBL production by Kleb*siella spp*. in an urban hospital in South India.

*Methods:*Antimicrobial susceptibility testing of 213 strains of *Klebsiella spp*. isolated from various clinical specimens was carried out by disc diffusion method.One hundered and five strains found to be resistant to at least one of the third generation cepholosporins tested, viz, cefotoxime, ceftazidime and ceftrioxone were screened for the production of ESBL by phenotypic and genotypic methods.The phenotypic methods include the phenotypic confirmatory disc diffusion test (PCDDT)and double disc diffusion synergy test(DDST)and the genotypic method include the PCR amplification method.

*Results:*Of the 105 strains screened for ESBL producton 45(42.85%)and 43(40.95%) strains were positive by PCDDT and DDST methods respectively and 53(50.47%)strains were positive by genotypic method.

*Conclusion:*The prevalence of ESBL produced by *Klebsiella spp*. from various clinical conditions in this part of the country is 24.88% as detected by the genotypic method.The study suggests that ESBL production should be detected routinely to monitor the resistant organisms for implementation of appropriate infection control measures.

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17.020

A Novel Extended-Spectrum SHV-Type Beta-lactamase, SHV-104, From *Klebsiella pneumoniae*

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Background: Since first reported in Germany in 1983 and in the United States in 1989, extended-spectrum β-lactamases (ESBLs) have spread worldwide. These enzymes are mostly plasmid-encoded derivatives of TEM-1, TEM-2, and SHV-1 by one or more base pair changes or are from a rapidly evolving class called CTX-M.

Methods: K. pneumoniae ML2011 was collected on July 2004, from intensive care unit of Military hospital in Tunisia. Identification of strains was performed by using both API 20 E and the VITEK automated system. Minimal inhibitory concentrations (MICs) were determined by E-test Strips for the strain on Mueller-Hinton agar as recommended by the Clinical and Laboratory Standards Institute (CLSI) (CLSI/National Committee for Clinical Laboratory Standards [NCCLS], 2006). Transfer of resistance phenotypes was performed by transformation and conjugation experiments. The ESBL was identified by double-disk synergy test, by isoelectric focusing and sequencing of PCR products.

Results: MICs for *K. pneumoniae* ML2011 showed that this strain was resistant to all β-lactams tested expect imipenem. *K. pneumoniae* ML20011 exhibited a high level of resistance to oxyimino cephalosporins. The strain was also resistant to kanamycin, chloramphenicol, ciprofloxacin, nalidixic acid, tetracycline and streptomycin. The disk diffusion method showed synergy between ceftazidime, cefotaxime, aztreonam, ceftriaxon, and amoxicillin-clavulanic acid against the strain and its transformants and transconjugants, suggesting plasmid-mediated production of an ESBL enzyme. PCR analyses confirmed the presence of blaSHV in parent strain *K. pneumoniae* ML2011, and its transformants *E. coli* DH5α/pML2011 and transconjugants *E. coli* HB101 X pML2011 indicating that this gene is located on transferable plasmid with estimated molecular size of 50 kb. Nucleotide sequence was performed on the coding region 861pb used to predict the amino acid sequence. This sequence was compared with strain *K. pneumoniae* Kp297 (DDBJ/EMBL/GeneBank accession no. EF035567) for nucleotide sequence homology and predicted amino sequence. Two amino acid substitutions were found at position 5 and 202, resulting respectively in a Met (ATG) to Leu (TTG) and an Arg (CGT) to Ser (AGT) changes. As theses substitutions not showed by other known SHV β-lactamases, the pI 7.3 from *K. pneumoniae* ML2011 appears to be a novel ESBL and has been designated SHV-104 (http://www.lahey.org).

Conclusion: The modification at position 202 may result in a change of the pI to 7.3 and the extended spectrum of the enzyme.

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17.021

Highly Susceptible Strains of Typhoid Bacilli Encountered in Jamaica

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Background: Unlike reports of multidrug-resistant *Salmonella enterica serovar typhi* (*S. typhi*) in countries around the world, strains encountered in Jamaica have been uniformly susceptible to all the anti-typhoid drugs and also to other antibiotics by disc method. We have been maintaining these isolates on Dorset-egg medium over the years. In this report, we examine the MICs of 4 front-line antibiotics against 41 unduplicated isolates(one from a patient) encountered in consecutive 17 years between 1984 and 2000 at the University Hospital in Kingston, Jamaica.

Methods: The MICs were determined by E test(AB Biodisk, Solna, Sweden) using *E. coli* ATCC 25922 as control. Manufacturers' instructions in regard to media, inoculum density and incubation parameters were followed strictly. Our observation of extremely low MICs (see results below) made us to do the tests repeatedly and read the results independently by each of us (NCB and OH) and repeat again if we differed in reading by more than one E test dilution.

Results: The MICs(μg/ml) of the four antibiotics were Chloramphenicol MIC range 2—4, MIC_{50} 3 and MIC_{90} 3; Ampicillin MIC range 0.125—1, MIC_{50} 0.25 and MIC_{90} 0.5; Trimethprim/Sulpha MIC range 0.023—0.064, MIC_{50} 0.032 and MIC_{90} 0.047; Ceftriaxone MIC range 0.023—0.047, MIC_{50} 0.032 and MIC_{90} 0.047. All isolates were susceptible. MICs were extremely low, fell in a narrow range and far below the standard susceptible(CLSI) Breakpoint MICs of the antibiotics. We have not seen any report of such a highly susceptible strains of typhoid bacilli from anywhere in the world.

Conclusion: Considering the growing increase of multidrug-resistant typhoid in countries around the world and reports of isolation of strains with MICs of front-line antibiotics of more than 256 μg/ml(Hirose K et al Antimicrob Ag Chemother 45:956—958,2001), the highly susceptible nature of strains encountered in Jamaica is noteworthy. These unique strains which we call 'Jamaica strains' have been persisting in this island country throughout the years.

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17.022

Carbapenem Resistance Mechanisms in *Acinetobacter spp*. Isolated from University of Malaya Medical Centre (Ummc)

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Background: Carbapenem-resistant *Acinetobacter spp*. have gained increasing significance as opportunistic pathogens in hospitalized patients. Carbapenem resistance is often associated with the loss and/or decrease in outer membrane protein and overexpression of multidrug efflux systems. In this study, we describe a study on carbapenem resistance mechanisms involved in *Acinetobacter spp*. isolated from UMMC.

Methods: 39 carbapenem-resistant clinical isolates of *Acinetobacter spp*. obtained from inpatients at the University Malaya Medical Centre were used in this study. Preliminary screening for carbapenemase production was carried out and IEF was determined in the strains. The isolates were analyzed for the presence of the blaIMP gene using PCR and confirmed by Southern hybridization to obtain the location of this gene. Other resistance mechanisms such the presence of AdeABC efflux pump genes were also determined by PCR followed by inactivation by plasmid insertion and the resultant mutants were tested for their antimicrobial susceptibilities. Presence of outer membrane proteins were determined by SDS-PAGE. Iron-regulated outer membrane proteins (IROMPs) were expressed under iron deficit conditions as these are possible targets of antimicrobial therapy. Thus, antibodies against these IROMPs were raised and bactericidal activity of the strains was determined.

Results: Out of the 39 strains only two strains, S26 and S90, both *A. calcoaceticus* were positive for the presence of metallo-β-lactamases. Both these strains had similar MIC values for imipenem, cefotaxime, and aztreonam at 32, 512, and 64 μg/ml respectively. IEF analysis showed that both strains had a band of pI 8.0 which corresponded to that of blaIMP-4, while an additional band of pI 7.0 was present in strain S90. Strains, S26 and S90, were PCR positive for blaIMP, while the remaining 37 harbored blaOXA-23. Amplification and subsequent nucleotide sequencing of the entire coding region of blaIMP confirmed the identity of the blaIMP amplicon to be blaIMP-4. Plasmid analysis revealed that only the two strains, S26 and S90, carried plasmids: 147, 63, 36 in both strains with an additional 7 kb plasmid in S26. Southern blot hybridization showed that the blaIMP-4 gene was located on the 36 kb plasmid in strain S26 and was confirmed to be located on Class 1 integron. Screening and nucleotide sequencing of the Class 1 integron revealed identical genes: blaIMP4, qacG, aacA4, and catB3 in the 2 strains. However, PFGE analyses showed that S26 and S90 had different genotypes. Screening of efflux pump genes showed that 36 strains harboured all the 3 genes (adeA, adeB, and adeC). Inactivation of these individual genes showed decreased antimicrobial susceptibility indicating its contribution towards the development of antimicrobial resistance. Besides that, all the strains showed loss of a 27 kDa OMP. The monoclonal antibodies produced showed bactericidal effect against the organism tested and it specifically killed